Molecular diagnosis of genital *Mycoplasma and Ureaplasma species* by duplex PCR and Real-time PCR in symptomatic and asymptomatic women attending a tertiary care hospital in Puducherry

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1. INTRODUCTION

Infertility can be due to many causes, but infectious agents serve as important agents for female infertility include *Neisseria gonorrhoeae, Chlamydia trachomatis, Treponema pallidum and Mycoplasma species*, which come under sexually transmitted pathogens cause sexually transmitted diseases (STD) threaten the reproductive health worldwide [1]. Globally infertility is considered as a serious public health concern which affecting 22 million women of which 56% are within the age of 15 -45 years [1]. The Ureaplasma and Mycoplasma species is peculiar to bacteria because of their tiny size and absence of cellular wall. Their unique shape and these factors position them at the family level of Mycoplasmatacae and belong to the class Mollicutes. They lack cell walls and small genome size in which they are unable to be affected by products such as penicillin that aim at inhibiting cell wall formation. [2]. Ureaplasma species are also known to be pleomorphic in nature [3]. The organisms belonging to this group of bacteria were at one time thought to be part of Mycoplasma, but based on characteristics like their urease activity and their ability to grow on media containing urea they were deemed different [4-5]. In 1954, Shepard together with other researchers cultured the initial strains from the human urogenital tract, hence the development of the presence of the Ureaplasma genus [3]. Ureaplasma species are now seen as substantial agents of pathogenicity pertaining to urogenital infection as well as problems associated with pregnancy and neonatal status [5-7]. Among them, the most important for their pathogenicity are the ability to adhere to epithelial cells in the urogenital tract due to the action of surface adhesins [5]. Moreover, low-grade infection can be maintained through two mechanisms; one of them is the antigenic variation in which the Ureaplasma surface proteins change, asking for immune surveillance. Ureaplasma also produces several cytotoxins and enzymes such as phospholipases that mediates the destruction of host cell membranes and initiates inflammation [10]. Since mycoplasma grows slowly and the lack of earlier

diagnostic tools infertility has been overlooked. Ureaplasma and Mycoplasma may be a very common infection that is mainly spread via sexual contact [5]. It seems to be threatening bacteria to cause infections in reproductive health and in fertility. There are many things that can cause infertility in women with social problems, psychologically affected and clinical depression, etc [11-12]. Moreover, Ureaplasma infection that occurs in the uterus when comes from the lower genital tract and reaches the amniotic fluid and placenta puts at risk the health of the mother and fetus [12-13]. The presence of *Mycoplasma* in placentas is associated with chorioamnionitis, low birth weight, and a significantly higher rate of spontaneous abortion, prematurity, perinatal morbidity, and mortality [6]. The culture of *Mycoplasma genitalium* can take up to 4 weeks, and the medium itself is complicated and costly. The Mycoplasma species were recovered from the urine samples by using SP-4 medium [14]. Demonstration of antigens/DNA of these pathogens in the clinical specimens is less time consuming and simpler than culture [15]. The availability of commercial multiplex PCR kits to target all these seven pathogens simultaneously is advantageous for researchers carrying out the prevalence studies of these diseases, but this commercial kit is to be imported and quite expensive [16]. Classification of the organisms in this genus has changed with time due to the availability of applied molecular epidemiological tools that help clarify their phylogenetic status. Duplex PCR is a variation of PCR that allows the simultaneous amplification of two different gene targets in the same reaction mixture. This technique is useful for comparing two genes or detecting a target gene and a reference gene simultaneously. The main purpose of the study is to detect the prevalent genotypes of Ureaplasma and Mycoplasma species from symptomatic and asymptomatic women in and around Puducherry state.

2. AIM & OBJECTIVES

Aim

- To identify the prevalent genotypes of *Mycoplasma* and *Ureaplasma* species from symptomatic and asymptomatic women in and around Puducherry.

Objectives

- To detect the genes of Mycoplasma and Ureaplasma in the urogenital secretions of both symptomatic and asymptomatic women by using duplex PCR and Real Time PCR.
- To perform Gene sequencing for the amplified products of Mycoplasma and Ureaplasma species.

3. RESEARCH QUESTIONS

- What is the prevalence of Mycoplasma, Ureaplasma in and around Puducherry?
- What are the predominant genotypes of Mycoplasma and Ureaplasma species present in and around Puducherry?

4. BRIEF REVIEW OF LITERATURE

Global Prevalence:

Nowadays, the common knowledge about vagina contains a significant amount of bacteria, even in healthy normal women. The number of isolates gradually increased after then, including Gram positive cocci (*Streptococcus pyogenes, Staphylococcus albus*) and Gram-negative bacilli (*E. coli, Klebsiella sp, and Proteus sp*) in developed as well as developing nations, prevalent pathogens are associated with sexually transmitted diseases (STDs) specifically *Ureaplasma urealyticum* (UU), *Mycoplasma hominis* (MH), *Mycoplasma genitalium* (MG), and *Chlamydia trachomatis* (CT). Approximately 340 million individuals globally have been diagnosed with sexually transmitted infections (STIs) [1]. Prevalence rate of *Mycoplasma hominis, Ureaplasma urealyticum* and *Mycoplasma genitalium* in infertile women by Multiplex real-time quantitative PCR in Iran is 16%, 46% and 16.5% respectively [17]. In patients with nongonococcal urethritis, the prevalence of MG varies from 13% to 42% [16]. Species of *Mycoplasma* and *Ureaplasma* pose a global danger to fertility and reproductive health. The concurrent roles of these substances in infertility have been ignored due to a lack of sensitive, accurate, and reasonably priced diagnostic instruments. Rapid detection and simultaneous identification of the *Mycoplasma* and *Ureaplasma* species by real-time PCR and melt curve analysis among fertile and infertile females [17]. Sequence analysis reveals asymptomatic infection with *Mycoplasma hominis and Ureaplasma urealyticum* possibly leads to infertility in females for *M. hominis, U. urealyticum* and got prevalence rate of 6.45% for *M. hominis* and 3.23% for *U. urealyticum* [18,19]. Eaton et al. (1944) identified Mycoplasma is a filterable virus as the causative agent of primary atypical pneumonia. Later on, it was determined that this was *M. pneumonia*, a type of pleuropneumonia-like organism, or PPLO. There are 200 species of Mycoplasma identified in reptiles, animals, plants and humans [20]. The pathogenic species of the Ureaplasma or

Mycoplasma genus in humans induce hidden infections that eventually lead to chronic illnesses [21].

National Prevalence:

The frequency of *Chlamydia trachomatis* (CT) and genital mycoplasmas in Indian patients are suffering from genital tract infections. A preliminary study was conducted and estimated the infection rate for Mycoplasma and Ureaplasma was 13.4% and 36.5% by using commercial real time multiplex PCR (FTD Urethritis plus) [16]. The suggested novel species *U. parvum* (biovar 1) and *Ureaplasma urealyticum* (biovar 2) account for the majority of human Ureaplasma isolates [18]. The prevalence of *Ureaplasma urealyticum* is more than *Mycoplasma hominis*, and least of *Mycoplasma genitalium*, in Indian women with lower genital tract infection [4, 18]. The PCR-detected prevalence of *M. hominis* (20%) was much higher than the culture-detected prevalence (3.6%). The overall prevalence of *M. genitalium, M. hominis*, and *U. urealyticum* was 11.73%, 11.33%, and 9.68%, respectively. Compared to men, women with genitourinary tract infections had a higher prevalence of these infections [2].

5. MATERIALS AND METHODS

Type of Study: Cross Sectional study

Place of study: Department of Microbiology, School of Allied Health Sciences, Sri Balaji Vidyapeeth, Department of Obstetrics and Gynecology, MGMCRI, Sri Balaji Vidyapeeth, And Mahatma Gandhi Medical Advanced Research Institute, Sri Balaji Vidyapeeth

Study Period: November 2020 to August 2024

Type of Sample: Endocervical swabs

Ethics approval

Approval was obtained from our Institutional Human Ethical committee (IHEC), MGMC & RI. Approval Project No: MGMCRI/RAC/2021/02/IHEC/12, dated 7th June 2021.

Inclusion Criteria

Patients presenting to Gynecology OPD with complaints of

- Pelvic inflammatory disease (PID), abnormal vaginal discharge, Burning micturition, cervical fragility, cervical erosions, genital ulcers and lower abdominal pain.

Exclusion Criteria

- Antenatal women with any known pregnancy complications
- Patients who have received macro bile/tetracyclines during the past 10 days.
- Women with cancerous or precancerous lesions of the cervix detected by clinical examination and/or Pap smear
- Age <18 years or > 60 years
- Ante-natal women in the third trimester

Sample size in each group (with justification)

Sample size required was calculated using following formula:

$$n = \frac{1.962(PQ)}{d^2}$$

P = Prevalence of the diseases

Q = (1-P)

d = Margin of error 0.06 (6%)

National Prevalence of symptomatic cases of 25.8% [Dhawan *et al*., 2012]

(i) Symptomatic women = 205

(ii) Asymptomatic women = 205

Total = 410

Specimen Collection and Processing:

After getting consent, the endocervical swabs were collected from the symptomatic and asymptomatic women. The swabs were collected to the tube containing 2ml of Phosphate Buffered Saline (PBS). Collected specimen was transferred immediately to the Research facility for DNA extraction and molecular analysis.

DNA Extraction:

DNA was extracted for a total of 410 samples as per the procedure of DNA Blood Mini Kit (Qiagen, Germany). The purity of the extracted DNA was checked by absorbance (A) using Eppendorf Spectrophotometer and the A260/A280 ratios observed in the range of 1.7-1.8 for all 410 samples. Finally, the aliquoted DNA was stored at −80°C till the completion of the molecular tests.

Duplex PCR:

In the present study, the Duplex PCR was carried out in Veriti Thermocycler (Applied Biosystems) for targeting of two different DNA and the primers of Ureaplasma species (*U.*

parvum and U. urealyticum) [22] and Mycoplasma species (*M. hominis and M. genitalium*) [23-24] were utilized from the study.

Standardization of Duplex PCR:

Conventional Gradient PCR was performed to set up an annealing temperature for each set of primers. Based on the gradient PCR, the common annealing temperature was applied for two different genes of Ureaplasma and Mycoplasma species. The same temperature was applied to the Duplex PCR for amplification and the patient PCR positive was taken as a positive control and no template DNA was taken as a negative control. Both positive and negative control was performed for each run.

Duplex PCR amplified products were analyzed by 2% agarose gel electrophoresis using Ethidium bromide-stained solution to visualize the bands under Gel Doc^{+} system (Bio-Rad, Germany) for the separation of the DNA fragments to separate and visualize the PCR products.

In-house Real Time PCR:

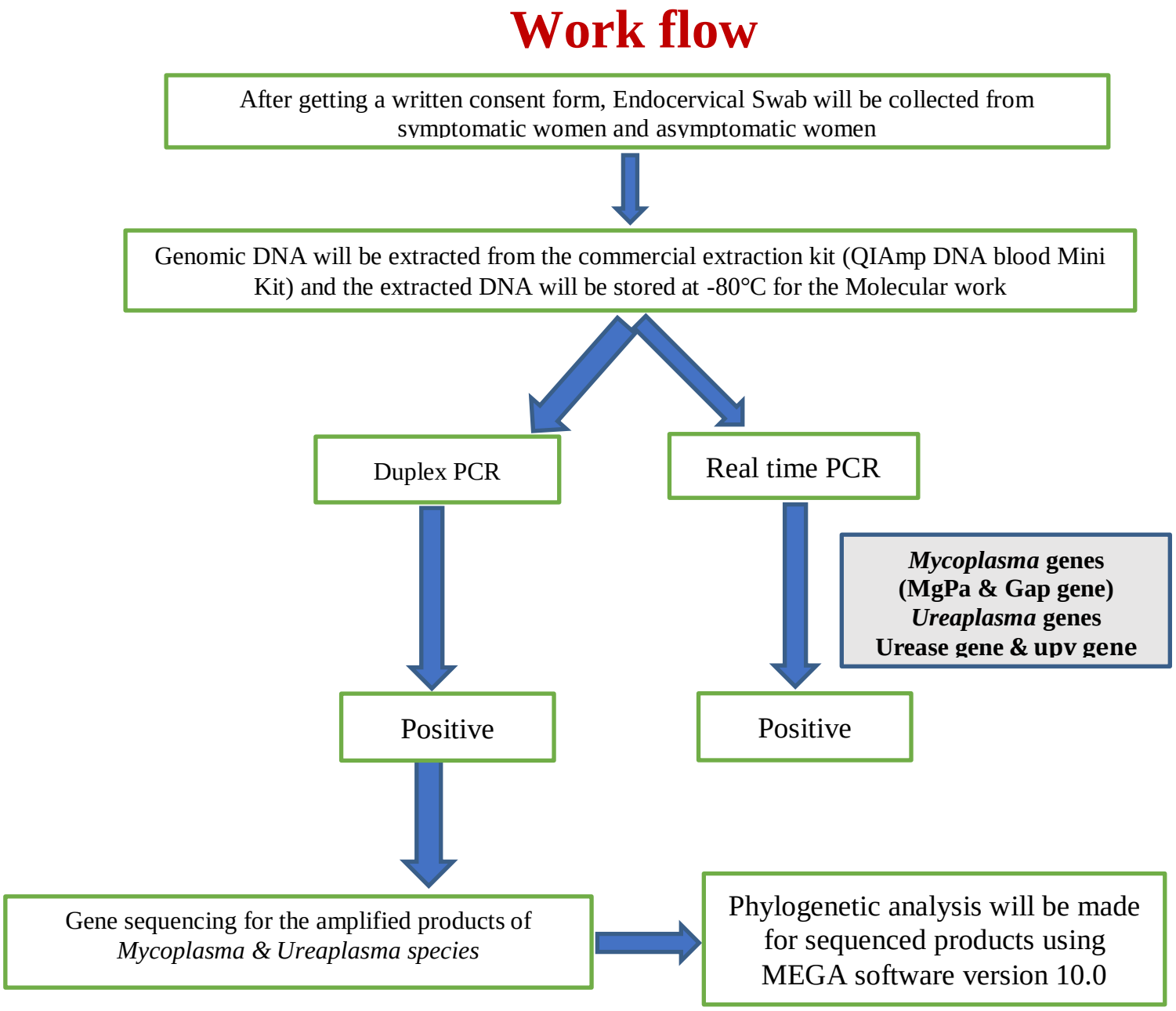
Real Time PCR was performed for a total of 410 samples targeting the four different genes in a Bio-Rad, CFX96 C1000 Touch machine. To amplify Urease and Upv genes for Ureaplasma and MgPa as well as Gap gene for Mycoplasma [23-24] was targeted. In each run a positive and negative controls were included. All samples were run in duplicates for confirmation of result. A Cq value of ≤ 35 was considered as positive. To ensure the quality of DNA, an internal control namely Human β – actin (ACTB) was used for every DNA sample.

Gene Sequencing:

In the present study, totally 13 (11 for Urease and Upv gene; two sequences for *Mycoplasma genitalium)* Duplex PCR products were purified using QIA quick PCR purification kit (Qiagen, Germany) as per the procedure outlined by the manufacturers. The

purified products were confirmed in a 2.0% agarose gel electrophoresis stained with Ethidium bromide. These amplicons were custom sequenced by means of bidirectional single pass sequence analysis by an Applied Biosystems, ABI3730XL.

Sample Processing Procedure


Work flow
After getting a written consent form, Endocervical Swab will be collected from symptomatic women and asymptomatic women
Genomic DNA will be extracted from the commercial extraction kit (QIAmp DNA blood Mini Kit) and the extracted DNA will be stored at -80°C for the Molecular work
Duplex PCR
Real time PCR
Mycoplasma genes (MgPa & Gap gene) Ureaplasma genes Urease gene & upv gene
Positive
Positive
Gene sequencing for the amplified products of Mycoplasma & Ureaplasma species
Phylogenetic analysis will be made for sequenced products using MEGA software version 10.0

6. STATISTICAL METHODS

Percentages will be calculated for categorical variables. Means and standard deviations (SD) will be calculated for numerical variables. Continuous variables will be compared using the student's t-test for normally distributed variables. The Chi-square test and Fisher's exact test will be used to compare the two groups using GraphPad Prism (Online software version). All P values < 0.05 will be considered statistically significant.

7. RESULTS

Demographic details of symptomatic and asymptomatic women

Mean ± SD age of the symptomatic women is 41.4 ± 11.3 (41.39 ±1.54) and asymptomatic women 41.2 ± 10.1 (41.2 ±1.38) with 95% confidence interval. These demographic details show that the average age of symptomatic women is slightly higher than that of asymptomatic women. Table – 1 represents the age-wise distribution of individuals in two groups: symptomatic and asymptomatic. These groups consist of 205 individuals each, making a total of 410. The distribution is across four age groups: 21-30 years, 31-40 years, 41-50 years, and 51-60 years. The highest proportion of symptomatic individuals is in the 41-50 age group (31.70%) followed by 31-40 years (29.2%). The highest proportion of age was observed in asymptomatic is 31-40 years (35.12%) followed by 41-50 years of age. There is no asymptomatic individual was found in the 51-60 age group. A statistical significance was observed between symptomatic and asymptomatic groups across 51-60 years and other age categories were not statistically significant.

Results for overall positivity of Ureaplasma & Mycoplasma infection in symptomatic and asymptomatic women

Table – 2 shows a total of 91 out of 205 symptomatic patients (44.4%) were positive for Ureaplasma, Mycoplasma, or both and 34 (16.6%) were positive for asymptomatic group. Ureaplasma was found 26.8% positivity in symptomatic patients followed by 10.7% in

asymptomatic women. Mycoplasma positivity was observed in 8.8% of symptomatic patients and 5.8% of asymptomatic women. Both Ureaplasma and Mycoplasma positivity was detected 8.8% in symptomatic patients, hence no positivity was observed in asymptomatic women. The main findings of the study showed that the asymptomatic women also had quite number of positive for both Ureaplasma and Mycoplasma species.

Table – 1: Age-wise distribution of Symptomatic and Asymptomatic Patients (n=410)

S.no	Age-wise distribution	Symptomatic (n= 205)	Asymptomatic (n=205)	Total (n=410)	P value*
1	21-30 years	51	61	112 (27.3%)	0.528
2	31-40 years	60	72	132 (32.2%)	0.204
3	41-50 years	65	81	136 (33.2%)	0.529
4	51-60 years	29	-	29 (7.1%)	**< .00001**

*p≤0.05 considered as statistically significant

Table – 2: Results for overall positivity of Ureaplasma & Mycoplasma infection in symptomatic and asymptomatic women (n=410)

Bacteria	Symptomatic (n=205) (%)	Asymptomatic (n=205) (%)	Total Positivity (n=410)
Ureaplasma	55 (26.8%)	22 (10.7%)	77(18.78%)
Mycoplasma	18 (8.8%)	12 (5.8%)	30(7.31%)
Both *Ureaplasma & Mycoplasma*	18 (8.8%)	-	18(4.39%)
Total Positivity	**91 (44.4%)**	**34 (16.6%)**	**125 (30.48%)**

Clinical conditions for symptomatic patients

Figure – 1: shows the clinical details for 205 symptomatic patients, Lower abdominal pain was the most predominant symptom 38 (18.53%) followed by heavy bleeding during menstrual cycle and abnormal bleeding 26 (12.68%) and 28(13.65%) cases respectively. Symptoms like dysmenorrhea and dysuria are reported, only in two (0.97%) cases each. Vaginal discharge and related symptoms like vaginitis with odor or vaginal discharge with odor also have a considerable number of cases.

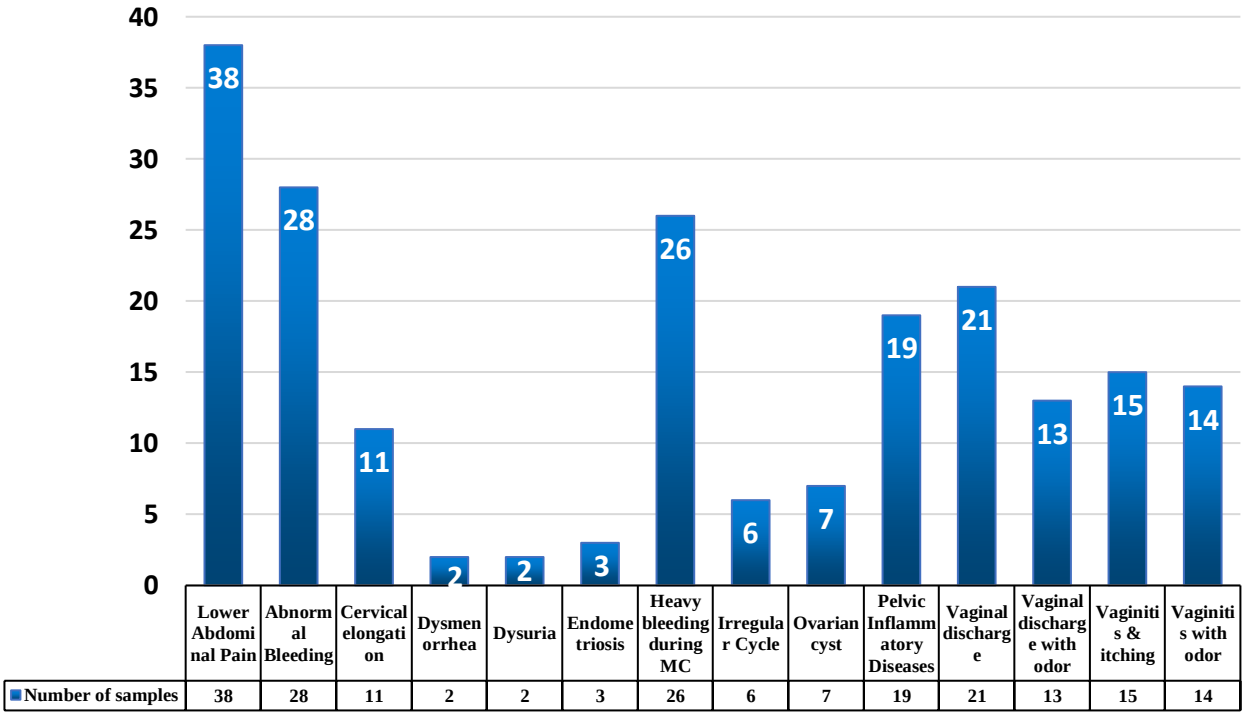


Figure – 1: Clinical details for symptomatic patients (n=205)

Results for Duplex PCR positivity on Ureaplasma & Mycoplasma species

Duplex PCR was positive for Ureaplasma is 24(11.7%) out of 205 symptomatic patients for both genes which showed in Figure – 2 followed by 17(8.29%), for only Urease gene, and 11 (5.36%) for the Upv gene. Out of 205 asymptomatic patients, 3(1.46%) tested positive for the Urease gene, but none for the Upv gene or both genes.

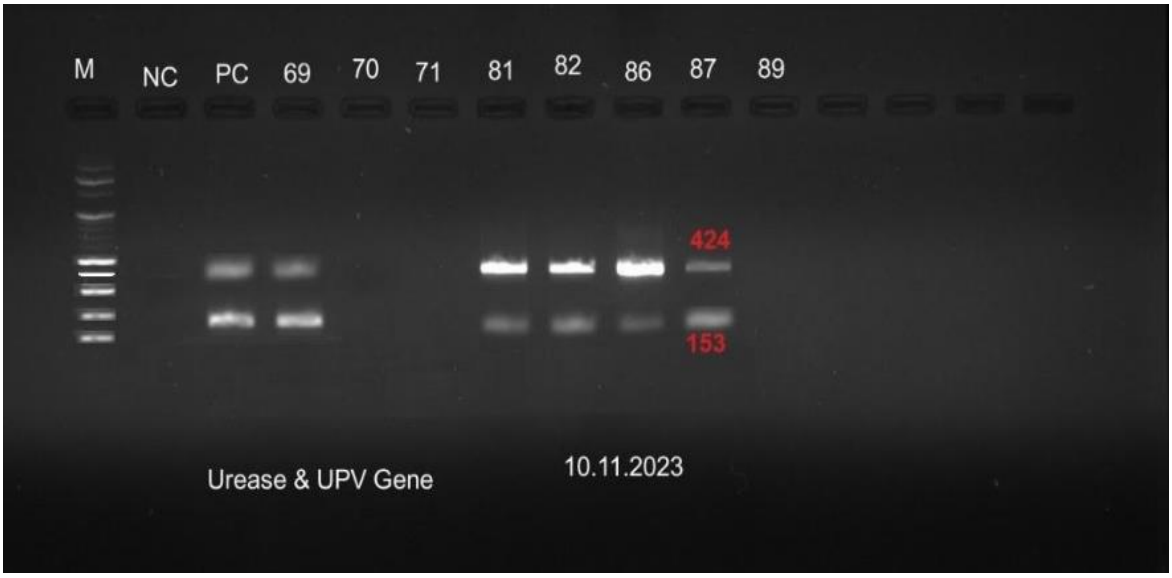


Figure - 2: Urease Gene & Upv gene positive in Duplex PCR

The gel electrophoresis shows Lane 81, 82, 86 & 87 was positive for genes, urease and UPV at 424bp and 153bp respectively. While the other samples from Lane 70, 71 & 89 shows negative for the targets. NC – Negative control; PC – Positive control and M – 100bp DNA ladder

In Mycoplasma positivity, 11 (5.36%) were positive for the MgPa gene, 13 (6.34%) for gap gene, and five for both genes. There is no positive for asymptomatic women and the results shown in Figure - 3.

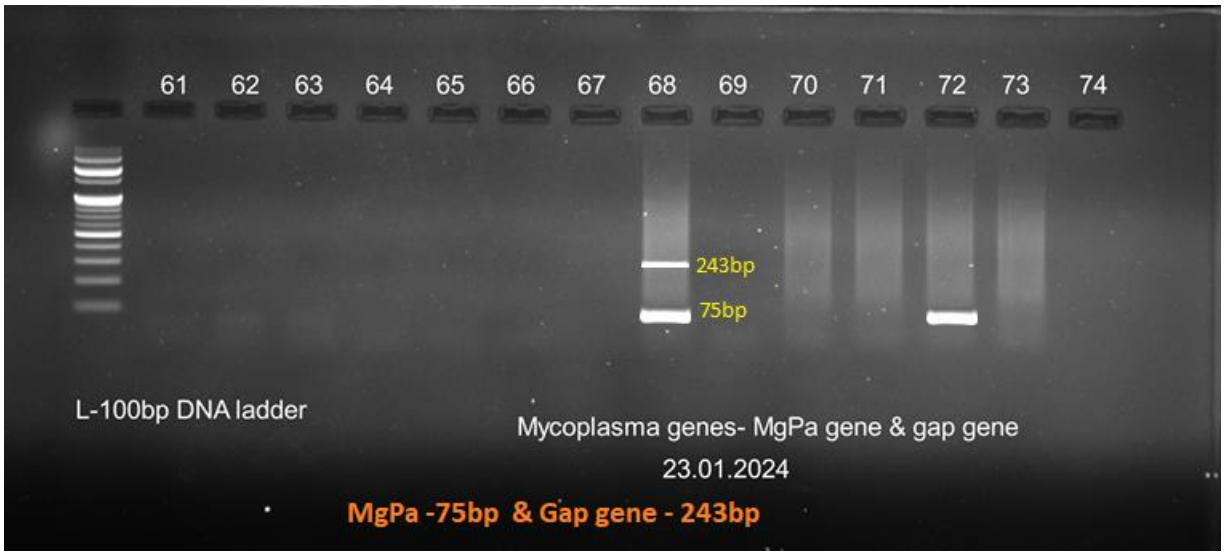


Figure - 3: Mycoplasma MgPa & Gap gene positive in Duplex PCR

The gel electrophoresis shows Lane 68 was positive for both MgPa and Gap gene at 243bp and 75bp respectively. While the other samples from Lane 72, shows positive for MgPa gene

and remaining lane 61 to 67 & 70, 71 shows negative for the targets. Lane 74 – Negative control and Lane L – 100bp DNA ladder

Results for Real time PCR positivity on Ureaplasma & Mycoplasma species

Out of 205 symptomatic patients, 19(9.26%) tested positive for the Urease gene, 21 (10.24%) for the Upv gene, and 33(16.09%) for both genes. Out of 205 asymptomatic patients, 7(3.41%) tested positive for the Urease gene, 12(3.41%) tested positive for the Upv gene but none of both genes. The P value for the Urease gene alone is 0.000015, which is highly significant in asymptomatic patients whereas 0.06901 not significant in symptomatic patients

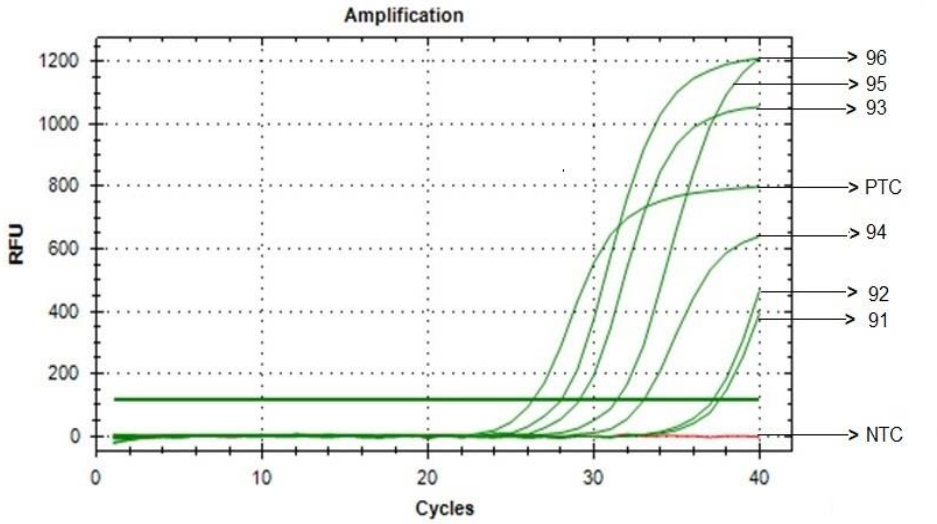


Figure – 4: Amplification curve for Ureaplasma Urease gene positivity. This qPCR amplification plot demonstrates that the samples (91-96) show varying degrees of target DNA amplification, with sample 96(Ct value 26.0) having the most target DNA and sample 91 having the least. The positive control amplifies at Ct value 26.0 and the negative control shows no amplification, indicating reliable qPCR results.

Out of 205 symptomatic patients, 14 (5.36%) tested positives for the MgPa gene, 15 (6.34%) for the gap gene, and 7 for both genes. Out of 205 asymptomatic patients, 4(5.36%) tested positives for the MgPa gene, 2(6.34%) for the gap gene, and 6 for both genes.

Ct value $\leq$ 35.0 was considered as positive for all four gene targets (urease, upv, MgPa & gap) and 28.0 ±2.0 for beta actin. After the completion of the dissociation curve, the melting temperature was noted as 84°C for urease, 84°C for upv, 84°C for MgPa & 84°C for gap gene. PCR products were confirmed by 2.0% agarose gel electrophoresis with Ethidium

bromide solution which showed a single band at 105bp, 100bp,120 & 90 for urease, upv, MgPa & gap gene respectively.

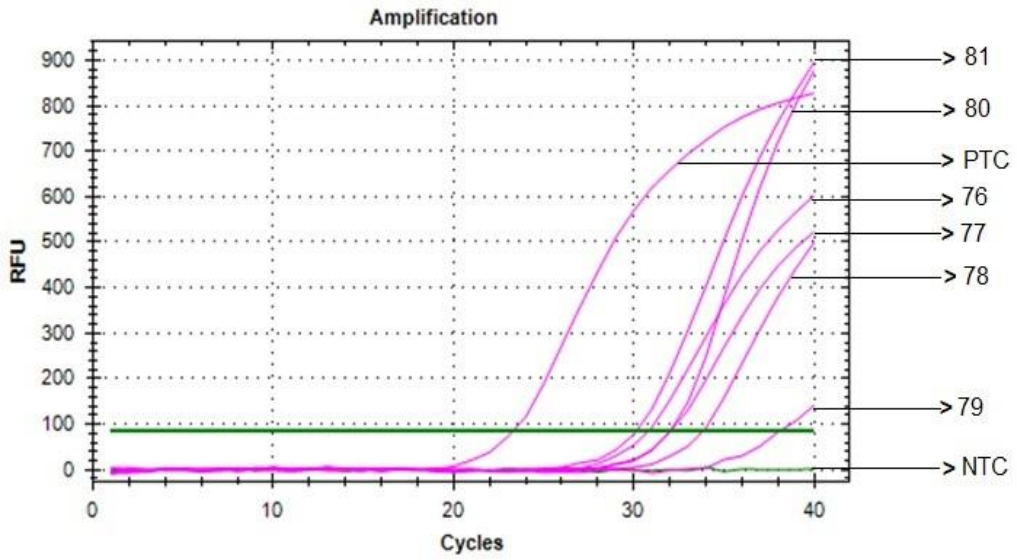


Figure –5: Amplification curve for Mycoplasma Gap gene positivity. This qPCR amplification plot demonstrates that the samples (76-81) show varying degrees of target DNA amplification, with sample 80 & 81(Ct value 30 & 31) having the most target DNA and sample 79 having the least. The positive control amplifies at Ct value 23.5 and the negative control shows no amplification, indicating reliable qPCR results.

Phylogenetic analysis of Ureaplasma species

The specificity of the 11 sequences was determined by using BLAST (Basic Local Alignment Search Tool) analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) showed maximum identity of 99-100% with other isolates available in NCBI database. These isolates were considered as reference strains for comparison of the identified sequences from isolates of the current study. The aligned 11 sequences PP153929 to PP153939 were available in the GenBank, NCBI database. The phylogenetic tree was constructed based on their genetic sequences obtained by the maximum likelihood method, which classifying Ureaplasma species from both genes as well as the genetic relatedness between various isolates of the same species which was illustrated in Figure – 4. Study isolates PP153939 and PP153938 forms were closely related with Iraq strain ON037201.1 which gives 100% bootstrap value. Four reference strains ON755316.1 and ON755318.1 from Baharain followed by

OM362258.1 and OM362276.1 from Iraq forms a separate ancestral lineages from other isolates. Study sequences pairs PP153933-PP153937, PP153936-PP153932, PP153935-PP153934, and PP153931-PP153929 shared a common ancestor with the cluster formed by Iran isolate MW076224.1, with bootstrap value 80.75%. Other reference strains from China, Australia and Iraq AB013929.1, AB014054.1, AF085731.2, AF085732.1 and OR775458.1, OR775459.1 are closely grouped with our study strains PP153930. The percentage of sequence similarity was 95.0% covered by reads with the sequences from reference strains.

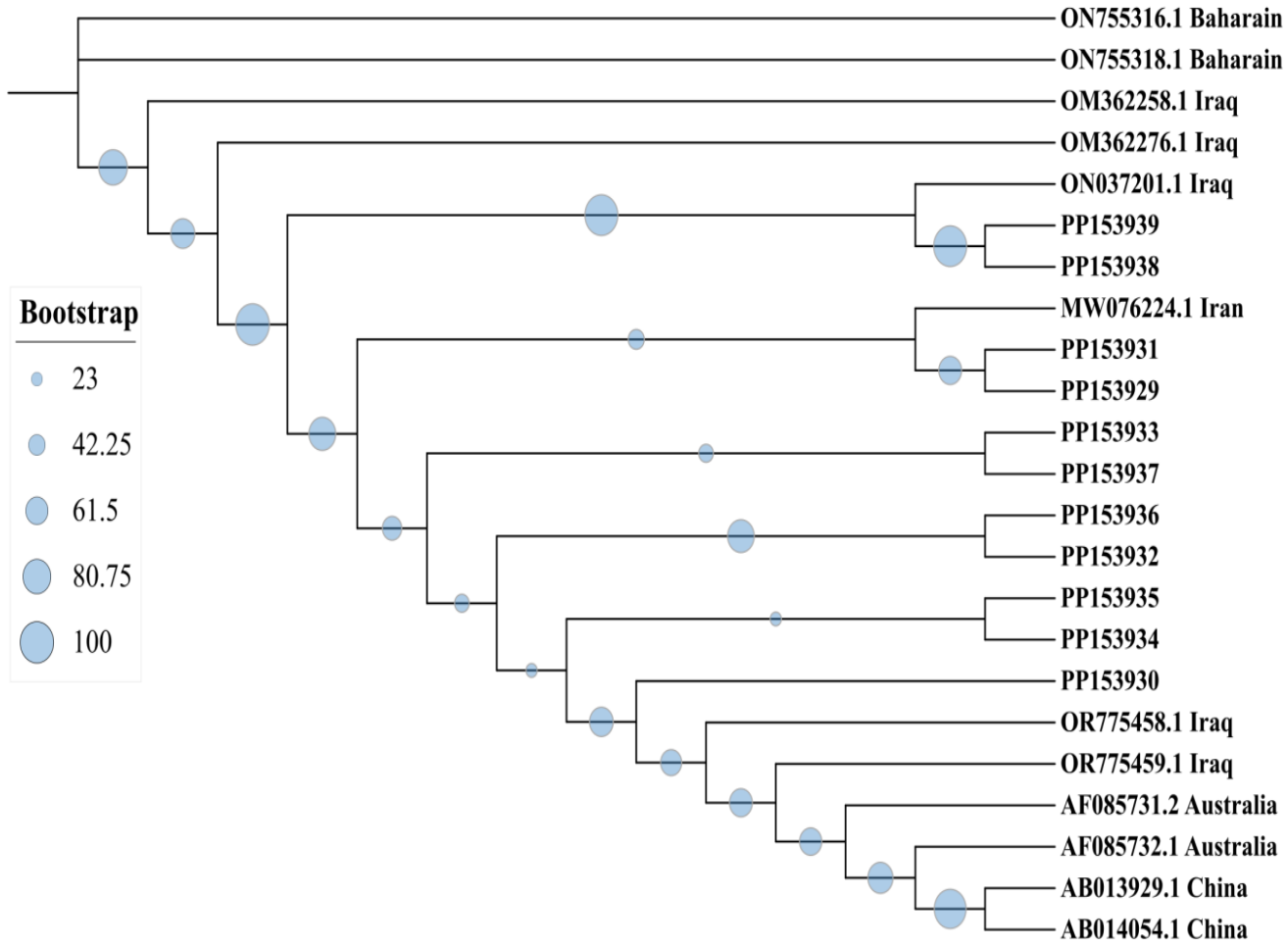


Figure – 4: Phylogenetic analysis of Ureaplasma species

8. DISCUSSION

Culture is the gold standard for determining Ureaplasma and Mycoplasma pathogen identities, yet this method has limitations in terms of contamination and time commitment [1, 13]. *Ureaplasma spp* and *Mycoplasma spp.* is a self-replicating organism, appears both in genome size as well as in cellular dimensions. *Ureaplasma urealyticum and Ureaplasma parvum* are the two important pathogens causing urethritis/cervicitis in infertile patients [10]. In compliance of guidelines for Sexually Transmitted Infections Treatment 2021, urethritis is known to cause by *Ureaplasma species* [1]. In India, New Delhi has conducted a prospective study using Multiplex PCR and culture for the detection of Ureaplasma species in 38 of 147 (25.7%) by Dhawan et al., 2012 [25]. Another study from the same research in the year 2006 found that the performance of culture and polymerase chain reaction *U. urealyticum* 32% and 45% respectively [20]. The present study prefers Duplex PCR than culture, due to more time consuming and overgrowth of other bacterial contamination [8]. Iranian study was showed the prevalence of Mycoplasma and Ureaplasma in pregnant women was 5.6% and 11.2%, respectively [8]. The present study, finds that 10.7% & 5.8% of asymptomatic women was positive for *Ureaplasma sps* and *Mycoplasma sps*. Since its quite higher when compared to the other studies prevalence 6.45% and 3.23% from Ezeanya-Bakpa CC et al., [19]. Incidence of Ureaplasma positivity was observed 30% and 14.3% in asymptomatic women among Nigerian population [25]. Globally, many researchers have found that *U. parvum* is the predominant isolates in cervico-vaginal samples in both fertility as well as infertility groups [5]. *U. urealyticum* infection affected majority of the Morocco population [27]. In Puducherry, Multiplex Real Time PCR was performed and 17 were detected both *U. parvum and U. urealyticum* using cervical swabs by Stephen et al., 2019 [16]. Moreover, 100% of viability was not found in PCR and it may also detect some non-viable Ureaplasma cells in the clinical isolates [18]. Ideally, in duplex PCR, the amplification of both targets should

occur simultaneously. However, there can be instances where one target is amplified more efficiently or preferentially over the other due to various factors. To address preferential amplification, optimization of PCR conditions, primer design, and template concentrations may be necessary to ensure balanced amplification of both targets in duplex PCR [8]. The study isolate PP195967 were closely clustered with common ancestors of reference strains from Austria, Ireland, Canada and China. Remaining study sequences such as PP195970, PP195966, PP195969, PP195971 and PP195968 forms a separate ancestor lineage for the USA strain JQ901478.1.

9. LIMITATION OF THE STUDY

The study may not account for all potential confounding variables such as socio-economic status, sexual practices, or other underlying health conditions that could affect infertility or Ureaplasma infection. These unmeasured factors could influence the study results and the association between Ureaplasma species and infertility. Conducting the study at a single hospital might limit the diversity of the pathogens.

10. CONCLUSION

This study makes a significant contribution by performing the effectiveness of duplex PCR in identifying the Ureaplasma species and Mycoplasma species by providing valuable insights for clinical practice, including improved diagnostic methods and targeted treatment strategies for public health concern. By addressing these areas, we can improve patient outcomes and existing our knowledge on Ureaplasma and Mycoplasma infections and their impact on human’s health.

11. FUTURE RECOMMENDATIONS OF THE STUDY

In future our research will focus on validating infection in different populations based on these findings and exploring the role of pathogenesis of Ureaplasma and Mycoplasma infections in patients, as well as comparing the reliable diagnostic methods to enhance the management and understanding this type of infections.

To create awareness on *Ureaplasma* and *Mycoplasma* infections among children and women with >18 years of age in the rural as well as urban community to get better reproductive health

12. ABSTRACT

Introduction:

Infertility is a global public health concern, affecting 22 million women, with 56% aged 15-45. Infectious agents like *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Treponema pallidum*, and *Mycoplasma* species are significant contributors. These pathogens, including sexually transmitted diseases (STDs) pose a threat to reproductive health worldwide. *Ureaplasma* and *Mycoplasma* species are unique due to their small size and lack of cellular wall, making them difficult to inhibit. *Ureaplasma* species are pathogenic agents for urogenital infections and pregnancy and neonatal issues.

Aims & Objectives: To identify the prevalent genotypes of *Mycoplasma* and *Ureaplasma* species from symptomatic and asymptomatic women in and around Puducherry by using duplex PCR and Real Time PCR. To perform gene sequencing for the PCR amplified products of *Mycoplasma* and *Ureaplasma* species.

Materials & Methods: Endocervical samples were collected from 410 patients, both symptomatic and asymptomatic, using Phosphate Buffered Saline (PBS). Two methods were employed: Duplex PCR and Real-time PCR for detecting Ureaplasma and Mycoplasma. Positive samples were purified and underwent gene sequencing using MEGA software version 10.0 for further analysis and also construct Phylogenetic tree using IQ Tree tool.

Results & Discussion:

Ureaplasma positivity of 52(25.3%) observed by Duplex PCR and 73(35.6%) positivity shown by Real time PCR for symptomatic patients residing in Puducherry & neighboring Tamil Nadu. Mycoplasma positivity of 29(14.1%) observed by Duplex PCR and 36(17.5%) positivity shown by Real time PCR for symptomatic patients residing in Puducherry & neighboring Tamil Nadu. Ureaplasma positivity of 3(1.71%) observed by Duplex PCR and 19 (15.2%) positivity shown by Real time PCR for asymptomatic patients. Mycoplasma

positivity of 12(5.8%) positivity shown by Real time PCR and there is no positivity identified by Duplex PCR for symptomatic patients residing in Puducherry & neighboring Tamil Nadu. GenBank Accession Numbers for 11 sequences for urease & upv were published in NCBI database PP153929 and PP153939.These sequences had 99-100% maximum identity with other reference isolates from GenBank records. Phylogenetic tree has revealed that the study sequences are more similar with reference strains from Iraq, Iran, Bahrain, Australia and China.

Conclusion:

This study contributes significantly in assessing the efficacy of duplex PCR in identifying *Ureaplasma* and *Mycoplasma* species, offering valuable insights for clinical practice, including enhanced diagnostic methods and targeted treatment strategies for public health issues.

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14. RESEARCH PUBLICATIONS FROM THE STUDY

1. Lavanya P, Vivek Mandal N, Vinoj G, Ramya R, Rupal S, Balasubramanian M, Uma AN, Pradeep J. Performance of Conventional PCR-based assays for the specific detection of Ureaplasma urealyticum and Ureaplasma parvum in asymptomatic women using endocervical swab samples. Afr. J. Bio. Sc. 2024;6:12.

2. 6. Lavanya P, Vivek Mandal N, Vinoj G, Ramya R, Rupal S, Balasubramanian M, Uma AN, Pradeep J. Molecular diagnosis of genital *Ureaplasma urealyticum and Ureaplasma parvum* infection by duplex PCR in symptomatic women attending a tertiary care hospital. Journal of Clinical and Diagnostic Research **(Accepted for Publication)**

BASIC COURSE WORK

Participated and fulfilled the requirements as per University norms of the online workshop on "RESEARCH METHODOLOGY, BIO – STATISTICS AND PREPARATION OF SCIENTIFIC MANUSCRIPT" held at SBV from 10th March to 15th March, 2021 and secured 4 credit points.

Participated and secured A grade with 2 credit points in "UGC Mandate RESEARCH AND PUBLICATION ETHICS COURSE" held at SBV from 16th June to 21st July 2021.

CONFERENCES/CME/WORKSHOP ATTENDED

1. Poster Presentation on INFORM 22 by Panimalar Medical College Hospital & Research Institute on 22nd April 2022, entitled "Molecular detection of genital Mycoplasma genes among symptomatic women by using conventional Polymerase Chain Reaction – A pilot study" Awarded – Special Jury Award.

2. Paper Presentation- in 4th National Conference of Indian Confederation of Medical Laboratory Sciences ICLMS-2023 for Medical Laboratory Professionals on 30.09.2023 in JIPMER, Puducherry, and secured **"Best paper award** in the topic of "Molecular diagnosis

of genital Ureaplasma species in Symptomatic &Asymptomatic women by using Duplex Polymerase Chain Reaction"

3. Paper Presentation on NCMLT 2022 by Vinayaka Mission Research Foundation, Salem on 26th February 2022, Entitled "Analyzing the sources of PCR carryover contamination from DNA amplified products in Molecular Research laboratory.